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OBSERVATIONS ON STREPTOLYSIN

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As is well known, certain streptococci produce hemolysin (streptolysin), concerning the nature and mode of production of which, as well as its relation to virulence, there are differences of opinion.

Besredka[1] found that only those streptococci which were virulent for rabbits produced hemolysin, and he recommended that in the study of the hemolysin, cultures should be made directly from the heart blood of rabbits that are dead from streptococcus infection. Ruediger[2] found only a slight hemolytic property in the filtrates of streptococcus culture in heated rabbit serum after 24 hours. Schlesinger[3] was unable to obtain any production of hemolysin by streptococci that did not originally produce hemolysis, even when they had become virulent from passage through animals. Of 16 strains of streptococci Kerner[4] found that 11 produced hemolysis; he also noted that the production was more marked in cultures made directly from an animal than when artificial cultivation had been carried on for some time. M'Leod[5] asserts that there is a close association between the virulence and hemolytic power of certain streptococci. Landsteiner[6] was unable to obtain hemolysin except from a few streptococci and then only after they had been passed through animals. Sachs[7] does not agree with these investigators. His results indicate that there was no constant difference in the production of hemolysin by strains cultivated for some time and strains freshly isolated. He was unable to secure any filtrable hemolysin. Of the corpuscles of man, rabbit, dog, goat, sheep, beef, swine, guinea-pig, chicken and horse, those of man and rabbit were found the least resistant by Ruediger. He worked with a streptolysin which completely laked one drop of a thick suspension of washed corpuscles in 1½ hours. The corpuscles of chicken, beef and horse were somewhat more resistant than the other corpuscles mentioned, but as they could be made more susceptible by washing 5 times in salt solution, there seemed to be something in the serum of these animals that protect the corpuscles against the streptolysin, and he made careful tests of the antilysin. Besredka says that lysin produced by streptococci in human serum lakes human, rabbit, guinea-pig, goat and sheep corpuscles, but not chicken and goose; streptolysin produced in goat serum acted on human, rabbit and guinea-pig corpuscles, but not on goat, sheep, chicken and goose corpuscles, and he concludes that streptolysin and probably also the product of other bacteria may be different if produced in different mediums. Ruediger was unable to verify these results, streptolysin produced in goat serum, as well as that produced in human or

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[1] Ann. d. l'Inst. Pasteur, 1901, 15, p. 880.
[2] Jour. Am. Med. Assn., 1905, 44, p. 198.
[3] Ztschr. f. Hyg. u. Infektionskr., 1903, 44, p. 428.
[4] Centralbl. f. Bakteriol., I, O., 1905, 38, p. 223.
[5] Jour. Path. and Bacteriol., 1912, 16, p. 321.
[6] Centralbl. f. Bakteriol., I, Ref., 1906, 42, p. 785.
[7] Ztschr. f. Hyg. u. Infektionskr., 1909, 63, p. 463.

rabbit serum laking goat, sheep and chicken corpuscles; he found that the streptolysin produced in rabbit serum acted more quickly than the others. Braun[8] found that on blood agar streptococci may produce filtrable lysin, sometimes in large quantity, after a growth of from 8-10 hours. He found also that the lysin may be very sensitive and lose in strength after a few hours at 37 C.; the filtrate of the culture fluid was toxic for rabbits after cultivation for 10 hours but not for mice or guinea-pigs. In his experiments the most sensitive corpuscles were rabbit, human and guinea-pig; normal rabbit, guinea-pig, horse and human serum contained antistreptolysin. Kerner[4] observed that the corpuscles of the dog were more sensitive to streptolysin than those of man or frog.

[8] Centralbl. f. Bakteriol., I, O., 1912, 62, p. 383.

The question arises as to what relation there may be between the production of streptolysin and the virulence of streptococci. Do the more virulent streptococci produce more lysin than the less virulent? If so, is this true under all conditions? Another question is the cause of the varying sensitiveness of corpuscles of different animals to the action of streptolysin. In order to throw light if possible on some of these questions, experiments have been made, the results of which I present.

THE RELATION OF THE PRODUCTION OF STREPTOLYSIN TO THE MEDIUM, TIME AND ACID FORMATION

In my experiments I have used a streptococcus from an abscess of the frontal sinus and cultivated artificially for about a year. It is hemolytic on blood agar and forms long chains in dextrose broth. After trial I found that animal serum or broth mixed with serum is the best medium for the production of streptolysin. Plain broth and rabbit serum in different proportions, the serum being first heated at 56 C. for half an hour and incubated at 37 C. for 24 hours to test its sterility, was inoculated with streptococci, and the development of lysin studied with reference to time and acidity. The corpuscles used were washed at least 3 times in 0.9% salt solution and suspended in such solution. The serum broth cultures when filtered through Maasen filters gave a light yellow, clear and absolutely sterile filtrate. The mixture of culture fluid or filtrate and blood corpuscles was placed at 37 C. for 2 hours, with occasional shaking, and then in the icebox, the result being read after 15 hours. In order to determine the acidity, 10 c c of the medium was added to 90 c c of distilled water and titrated with phenolphthalein and N/10 NaOH. The tests of the streptolysin have been made as shown in table 1, which gives the proportions of serum and broth as 1:2, 1:1, 2:1, 4:1. These are the best mixtures

for the production of streptolysin. We see that in 5 hours the production was rather scanty, but after 10 hours a sudden increase occurred which continued for some hours, but after 48 and 72 hours the production gradually decreased. Braun also found the lysin production marked after from 8-10 hours' cultivation, and he emphasizes that the lysin is sensitive and that at 37 C. the lytic power may be destroyed in 6 hours. Hellens[9] seems to have reached the same result. Sekiguchi,[10] testing the relation between lysin and acid production, found that the production of lysin begins and ends earlier than the production of acid, lysin reaching its climax in from 15-18 hours, the acid in from 24-48 hours.

NATURE OF STREPTOLYSIN

Does the lysin in cultures of certain streptococci in serum broth lake different blood corpuscles in the same degree? If not, why? Is there any difference in the lysin production of virulent and nonvirulent streptococci? As shown in table 2, the streptococcus as isolated (1) and without having been passed through any animals, acts in about the same way on the corpuscles of different animals except that guinea-pig corpuscles seem to be a little more resistant; after passing the streptococcus through rabbits (2) its lytic power for various corpuscles is stronger, more particularly for rabbit corpuscles; when the strain that had been passed through rabbits passed through mice (3), the lytic power for mouse corpuscles is noticeably increased in a specific manner; when now the streptococcus is passed through a guinea-pig (4), the lytic action on guinea-pig corpuscles is stronger than for other corpuscles. The same results were obtained with the filtrates of the different cultures, although not in such marked degree. The results show that the production of lysin by this streptococcus increases as virulence is increased by passage through animals, and that the nature of the lysin changes according to the animal, being strongest for the corpuscles of the animal through which the strain in question has been passed last.

HEMOLYSIS BY CULTURE CENTRIFUGATES, STREPTOCOCCUS EMULSION, AND CULTURE FILTRATES

The results of the previous work on the filtrability of streptolysin have been divergent. I have found that a filtrable streptolysin may be obtained, but its lytic power is less than that of the unfiltered culture

[9] Centralbl. f. Bakteriol., I, O., 1913, 68, p. 602.
[10] Jour. Infect. Dis., 1917, 21, p. 475.

TABLE 1

PRODUCTION OF STREPTOLYSIN IN BROTH WITH VARYING PROPORTIONS OF SERUM, WITH REFERENCE TO TIME AND ACID FORMATION

Quantity of Culture Fluid	Salt Solution 0.9%	5% Suspension Rabbit Blood	Lysis																			
			Serum 1, Broth 2					Serum 1, Broth 1					Serum 2, Broth 1					Serum 4, Broth 1				
			Ages of Cultures in Hours																			
			5	10	24	48	72	5	10	24	48	72	5	10	24	48	72	5	10	24	48	72
1.0	...	1.0	++	+++	+++	+++	+++	++	+++	+++	++	++	++	+++	+++	+++	+++	++	+++	+++	+++	+++
0.5	0.5	1.0	+	+++	+++	++	+	+	+++	+++	++	++	+	+++	+++	+	+	+	+++	+++	+++	+
0.25	0.75	1.0	+	++	++	+	+	+	+++	+++	+	+	+	+++	+++	+	+	0	+++	+++	+++	0
0.125	0.875	1.0	0	++	+	+	0	0	++	++	+	0	0	++	++	0	0	0	++	++	+	0
0.05	0.95	1.0	0	+	+	+	0	0	++	++	0	0	0	++	++	0	0	0	+	++	+	0
0.025	0.975	1.0	0	+	0	0	0	0	+	+	0	0	0	+	+	0	0	0	+	+	0	0
0.0125	0.9875	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	1.0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Degree of acid				0.9	0.9	1.1	1.2		0.9	0.95	1.1	1.2		0.8	0.9	0.9	1.3		0.9	0.85	0.9	1.2

++++ = complete hemolysis.
+++ = strong hemolysis.
++ = moderate hemolysis.
+ = slight hemolysis.
± = trace of hemolysis.
0 = no hemolysis.

The streptococcus used in this experiment was obtained originally from the frontal sinus and the cultures used here were of a strain that had been passed through five rabbits and then through nine mice; the culture fluid in this case was not filtered.

TABLE 2

LYSIS BY UNFILTERED AND FILTERED CULTURE FLUID OF DESCENDANTS (1, 2, 3, 4) OF THE SAME STREPTOCOCCUS BUT SUBJECTED TO DIFFERENT TREATMENT

Quantity of Lytic Fluid	Salt Solution 0.9%	5% Suspension of Corpuscles	Human Corpuscles								Rabbit Blood							
			1		2		3		4		1		2		3		4	
			Fluid	Filtrate	Fluid	Filtrate	Fluid	Filtrate	Fluid	Filtrate	Fluid	Filtrate	Fluid	Filtrate	Fluid	Filtrate	Fluid	Filtrate
1.0	...	1.0	+++	++	+++	+++	+++	++	+++	+++	++	+	++++	+++	++++	+++	++++	+++
0.5	0.5	1.0	++	+	++	++	++	+	++	++	+	±	+++	+++	+++	+	+++	+
0.25	0.75	1.0	+	±	++	++	+	+	+	+	+	±	++	+	+	+	+	+
0.125	0.875	1.0	+	±	+	+	+	±	+	+	+	0	+	±	+	±	+	±
0.05	0.95	1.0	+	0	+	±	+	0	+	±	±	0	+	0	?	0	±	0
0.025	0.975	1.0	±	0	+	0	0	0	0	0	±	0	+	0	0	0	0	0
0.0125	0.9875	1.0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0
Control	1.0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Mouse Blood								Guinea-Pig Blood							
1.0	...	1.0	+++	+	++++	+++	++++	++++	++++	++++	+++	++	+++	+++	+++	+++	++++	+++
0.5	0.5	1.0	++	+	+++	+++	++++	+++	+++	+++	+	+	++	++	++	++	+++	+++
0.25	0.75	1.0	+	±	++	+++	+++	+++	+++	+++	+	0	+	+	+	+	++	+
0.125	0.875	1.0	+	±	+	+	++	++	+	+	+	0	+	±	+	0	+	0
0.05	0.95	1.0	+	0	+	±	+	+	+	+	0	0	0	0	+	0	+	0
0.025	0.975	1.0	±	0	+	0	+	±	+	±	0	0	0	0	0	0	0	0
0.0125	0.9875	1.0	0	0	?	0	+	0	0	0	0	0	0	0	0	0	0	0
Control	1.0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

fluid (table 3). A streptococcus culture in serum broth 1:1 was incubated for 10 hours; the particular strain had been passed through 5 rabbits and 18 mice. The culture was centrifugated at a rapid rate for about 1 hour, the sediment washed with 0.9% salt solution 3 times and resuspended in an amount of salt solution two-thirds that of the original culture. At the same time, the culture fluid was filtered through a Maasen filter. Table 3 shows that the filtrate and the centrifugate were lytic, the filtrate being less powerful, but the suspension of washed streptococci was without any lytic action.

TABLE 3

COMPARISON OF HEMOLYSIS BY CULTURE FILTRATE, CULTURE CENTRIFUGATE AND STREPTOCOCCUS SUSPENSION

Quantity of Lytic Fluid	Salt Solution 0.9%	5% Suspension of Corpuscles	Lysis		
			Filtrate	Centrifugate	Streptococcus Suspension
1.0	...	1.0	++++	++++	±
0.75	0.25	1.0	+++	++++	0
0.5	0.5	1.0	+++	+++	0
0.25	0.75	1.0	++	++	0
0.125	0.875	1.0	+	+	0
0.05	0.95	1.0	±	+	0
0.025	0.975	1.0	0	+	0
Control	1.0	1.0	0	0	0

RELATION OF SPECIFIC VIRULENCE AND PRODUCTION OF STREPTOLYSIN

As stated, a streptococcus after being passed through a given animal produces a lysin which seems to be somewhat specific for the corpuscles of that animal. The strain passed through 5 rabbits, 15 mice, and then through 7 guinea-pigs, gave results indicating that as the virulence for a particular animal increases, the power of specific lysin production also increases. I have found that this power may be retained, although with gradual weakening, over a period of 42 days through 7 generations on goat blood agar. In other words, when a streptococcus has acquired the power of producing lysin for a particular kind of corpuscle, that power may be retained for some little time. My results in general show, however, that unless the streptococccus is passed through animals at short intervals it does not produce much lysin. If cultivated directly from the heart blood of an animal that has died in consequence of its injection, the streptococcus is virulent, but if grown through several generations on blood agar the viru-

TABLE 4

HEMOLYTIC ACTION OF FILTRATE OF CULTURES OF STREPTOCOCCI AFTER PASSAGES THROUGH GUINEA-PIGS

Quantity of Lytic Fluid	Salt Solution 0.9%	5% Suspension of Corpuscles	After Passage Through 5 Rabbits and 15 Mice		Guinea-Pig Passages of Streptococcus after Passage Through 5 Rabbits and 15 Mice									
					1		2		3		4		5	
			Guinea-Pig Corpuscles	Mouse Corpuscles	Guinea-Pig Corpuscles	Mouse Corpuscles	Guinea-Pig Corpuscles	Mouse Corpuscles	Guinea-Pig Corpuscles	Mouse Corpuscles	Guinea-Pig Corpuscles	Mouse Corpuscles	Guinea-Pig Corpuscles	Mouse Corpuscles
1.0	...	1.0	+++	++++	++++	++++	+++	++++	+++	++++	++++	++++	++++	++++
0.5	0.5	1.0	++	++++	+++	+++	+++	++++	+++	+++	+++	+++	+++	+++
0.25	0.75	1.0	+	+++	+	+++	+	+++	++	+++	++	+++	++	+++
0.125	0.875	1.0	+	+	+	+	+	+	+	+	+	+	++	+
0.05	0.95	1.0	+	+	+	+	+	+	+	+	+	+	+	+
0.025	0.975	1.0	0	+	+	+	0	+	0	+	0	+	0	+
0.0125	0.9875	1.0	0	+	0	+	0	+	0	+	0	0	0	0
Control	0.1	1.0	0	0	0	0	0	0	0	0	0	0	0	0

lence is reduced while the production of streptolysin is not affected so much, as shown in table 5. After a longer time, however, of cultivation on blood agar, say 6-7 generations over a period of 42 days, the lysin production becomes reduced. The fact remains that virulence for animals and lysin production do not run strictly parallel. A non-virulent strain grown on artificial medium for a long time, however, is hemolytic still on blood agar.

TABLE 5

RELATION BETWEEN VIRULENCE AND PRODUCTION OF HEMOLYSIN

Generations of Streptococcus on Blood Agar	Virulence as Indicated by Fatal Dose for Mouse of 24-Hour Culture in Serum Broth	Production of Hemolysin. The lytic strength is indicated by the percentage of the corpuscle suspension, 1 c c of which was laked completely by 1 c c of the fluid of a 24-hour serum-broth culture	
		Mouse Corpuscles	Guinea-Pig Corpuscles
Original serum broth culture from blood of mouse....	0.00001 c c	5%	1%
First generation on blood agar.....	0.001 c c	5%	1%
Second generation on blood agar.....	.....	5%	1%
Third generation on blood agar.....	.....	5%	1%
Fourth generation on blood agar.....	0.05 c c	5%	1%
Fifth generation on blood agar.....	.....	5%	1%
Sixth generation on blood agar.....	.....	2.5%	1%
Seventh generation on blood agar.....	.....	2.5%	1%

THE EFFECT OF LYSIN ON CORPUSCLES AND QUANTITATIVE RELATIONS

It has been noted that a certain relationship must be secured between the lysin and the quantity of corpuscles in order to obtain the maximum lytic affect. I have tested the action of a fixed quantity of lysin on different corpuscles, and the results here also show that when a streptococcus is passed through a number of animals the lysin produced by that streptococcus is most active for the corpuscles of the last animal through which it passed.

TOXIC ACTION OF STREPTOLYSIN

According to Ruediger[2] and Braun, streptolysin is toxic for rabbits. I have found that 3-5 c c of lysin injected intravenously cause an attack of diarrhea with emaciation in rabbits; 1-2 c c injected into the abdominal cavity in mice would cause diarrhea lasting for several days, the animals in both cases recovering. Small quantities were without any apparent effect.

ABSORPTION EXPERIMENTS WITH STREPTOLYSIN

If the lysin produced by a streptococcus that has been passed through a certain animal has a specific action on the corpuscles of that animal, absorption tests with different corpuscles should reveal a similar specific relationship. Ruediger, Schlesinger and Hellens found that streptolysin combined with corpuscles at low temperature without causing lysin. I have made the following experiments bearing on this point:

To 20 drops of a thick suspension of mouse corpuscles were added 10 c c of streptolysin, the mixture kept at 0 C. for 5 hours and then centrifugated. The color of the fluid showed a trace of red. Similarly, another mixture was made with washed mouse corpuscles, and after centrifugation the corpuscles washed once more with 0.9% salt solution. On the addition of one drop of thick corpuscles suspension to 4 c c of the fluid of the first mixture, and after incubation for 2 hours and standing in the icebox for 15 hours, only a trace of hemolysis resulted. If one drop of the sediment of the second mixture was added to 4 c c of 0.9% salt solution, incubated for 2 hours and placed in the ice chest for 15 hours, marked hemolysis resulted. Untreated streptolysin caused complete lysis of mouse corpuscles in the control tests. This result shows that at low temperature there occurs a union between the lysin and the corpuscles, but no lysis. On injection of the clear fluid and the corpuscles in animals, the following results were obtained: In mice 1-2 c c of the clear fluid injected into the abdomen caused the same symptoms, with diarrhea, as the injection of filtrate of streptococcus culture. On the other hand, the injection of mice with 2-4 drops of mouse corpuscles treated with streptolysin, washed and then suspended in 2 c c of 0.9% salt solution, caused no symptom.

As these results indicate that the animals injected with fluid free from lysin developed the same symptoms as those injected with the untreated fluid, while the animals injected with the sediment remained well, some doubt is cast on the conclusion that the toxicity of streptococcus filtrates for certain animals is due to the lysin in such filtrates.

SUMMARY

The hemolysin production by streptococci is most successful in cultures in plain broth with equal or larger quantities of serum.

In such cultures the production of hemolysin is greatest after incubation at 37 C. for 10-24 hours; before 5 hours and after 48-72 hours only little lysin is produced.

There is a certain relation between the virulence of streptococci and the production of hemolysin. The production increases on successive animal passages as compared with the production in cultures of the same strain maintained on artificial medium.

The hemolysin produced by streptococci is filtrable, but it loses somewhat in strength on filtration.

Streptococci isolated from human beings and grown on artificial mediums for some time produce hemolysin that has nearly the same effect on the corpuscles of different animals, but when a streptococcus is passed through a number of animals of the same kind, its hemolysin production is increased, and this increase is most marked for the corpuscles of the animals through which the streptococcus has been passed. It seems as if the nature of the hemolysin depends to some extent on the host. If a streptococcus strain is passed through two species of animals, the corresponding hemolysin will be more active for the corpuscles of the species that harbored the streptococcus last.

Streptolysin unites with the corpuscles which it lakes, and streptococcus filtrates may remain toxic for mice after removal of the streptolysin by absorption with corpuscles.